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ORIGINAL ARTICLE

Longer duration of obesity is associated with a reduction in urinary angiotensinogen in prepubertal children

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Abstract

Background We aimed to study the impact of obesity on urinary excretion of angiotensinogen (U-AGT) in prepubertal children, focusing on the duration of obesity and gender. Also, we aimed to evaluate whether plasma angiotensinogen (P-AGT) and hydrogen peroxide (H_2O_2) play a role in the putative association.

Methods Cross-sectional evaluation of 305 children aged 8–9 years (160 normal weight, 86 overweight, and 59 obese). Anthropometric measurements and 24-h ambulatory blood pressure monitoring were performed. Angiotensinogen (AGT) was determined by a commercial enzyme-linked immunosorbent assay (ELISA) kit and H_2O_2 by a microplate fluorometric assay.

Results U-AGT and P-AGT levels were similar across body mass index (BMI) groups and between sexes. However, boys who were overweight/obese since the age of 4 years presented lower levels of U-AGT compared with those of normal weight at the same age. In children who were overweight/obese since the age of 4, urinary H_2O_2 decreased with P-AGT.

Conclusions A higher duration of obesity was associated with decreased U-AGT in boys, thus reflecting decreased intrarenal activity of the renin–angiotensin system. Also, children with a

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longer duration of obesity showed an inverse association between urinary H_2O_2 and P-AGT. Future studies should address whether these results reflect an early compensatory mechanism to limit obesity-triggered renal dysfunction.

Keywords Angiotensinogen · Obesity · Overweight · Body mass index · Children · Hydrogen peroxide

Introduction

The prevalence of childhood obesity has tripled in the last three decades, reaching epidemic proportions around the world [1]. Even at younger ages, obesity triggers a cascade of metabolic changes that contribute to the development of hypertension, diabetes, atherosclerosis, and chronic renal disease [2, 3]. Besides, obesity in childhood and adolescence predicts the risk of cardiovascular morbidity and premature mortality in adults [4]. The mechanisms underlying the association between a high body mass index (BMI) in childhood and adolescence and cardiovascular and kidney disease in adulthood are not well characterized, and no biomarkers are available for use in routine clinical practice to allow early identification of high-risk children.

The renin–angiotensin–aldosterone system (RAAS) has been implicated in the harmful consequences of obesity. This well-coordinated hormonal system regulates cardiovascular and renal function by controlling fluid and electrolyte homeostasis. Furthermore, tissue RAAS exists in specific tissues, namely the kidney and adipose tissue [5]. In the adipose tissue, RAAS components are highly expressed and secreted by mature adipocytes [6–8], although contradictory findings have been reported regarding its regulation in obese conditions. Some studies report an increased expression [6] and secretion [5, 9] of adipose tissue-derived RAAS components in obese individuals that might contribute to activate systemic RAAS and potentiate its deleterious effects [6, 8, 10, 11]. Other studies describe reduced expression of angiotensinogen (AGT) in adipose tissue that parallels the time course of adipocyte hypertrophy, which is tightly associated with the progression of obesity [12].

Childhood obesity is also associated with renal injury, and the kidney is particularly vulnerable to the effects of the RAAS, both through the hemodynamic impact of systemic RAAS activation and by local intrarenal activation, mostly derived from AGT produced in the proximal tubule [7, 13]. Urinary excretion of AGT (U-AGT) has been validated as a noninvasive biomarker of intrarenal RAAS activity [13] and increased U-AGT has been found in human hypertension, diabetes, and renal disease [13] even in adolescents [14, 15] and children [16]. The rise in U-AGT precedes the onset of microalbuminuria, suggesting that U-AGT might be an early marker of nephropathy [15]. Interestingly, excessive activation of the adipose RAAS seems to be crucial for establishment and progression of kidney disease [13, 17] but only two small sample studies have been performed in the setting of human obesity [7, 18]. In both studies, no difference was found in U-AGT between obese adults and nonobese controls. Remarkably, it was recently highlighted that not only severity but also duration of obesity can determine obesity-related target-organ lesions [19, 20] but neither of those studies took that in consideration. Also, the study of Thethi et al. showed that obese women had higher U-AGT than obese men, although there was no gender difference in the nonobese group [7]. Gender is known to influence blood pressure (BP) and cardiovascular and renal functions, and evidence implies that the RAAS might be a putative link in this association [21]. These data raise the possibility of gender differences in the association between RAAS and obesity.

The deleterious effects of intrarenal RAAS are due, at least in part, to potentiation of angiotensin II (Ang II)-mediated renal effects [2, 7], namely the production of reactive oxygen species and cytokines and the stimulation of cell growth, inflammation and fibrosis [13, 22]. Hydrogen peroxide (H_2O_2), a nonradical oxidant and a known mediator of Ang II effects [23], also appears to be involved in the regulation of RAAS components. Recent experimental studies suggest that H_2O_2 increases AGT expression in the kidney but induces its downregulation in adipose tissue [12, 24–26]. However, this relationship between H_2O_2 and AGT has not yet been explored in humans.

We aimed at characterizing the impact of obesity on U-AGT in prepubertal, otherwise healthy, children. We compared U-AGT between normal weight, overweight, and obese children and evaluated whether gender or obesity duration influence the putative association between obesity and U-AGT at this age. Furthermore, since experimental evidence indicates H_2O_2 as a modulator of AGT expression both in kidney and adipose tissue [12, 24–26], we evaluated the link between urinary excretion of H_2O_2 (U-H_2O_2), U-AGT, and plasma AGT (P-AGT) levels.

Methods

Study design and sample collection

We conducted a cross-sectional study of children aged 8–9 years followed since birth in a previously established Portuguese cohort study (Generation XXI) [27]. Children from the original cohort ($n = 8647$ newborns) were eligible for the ObiKid project if they had anthropometric data and a blood sample withdrawn at the study site in the 7-year-old evaluation (Fig. 1; $n = 4590$). For the Obikid project [28], we wanted to include a minimum sample of 300 children, since this sample size would provide a statistical power of

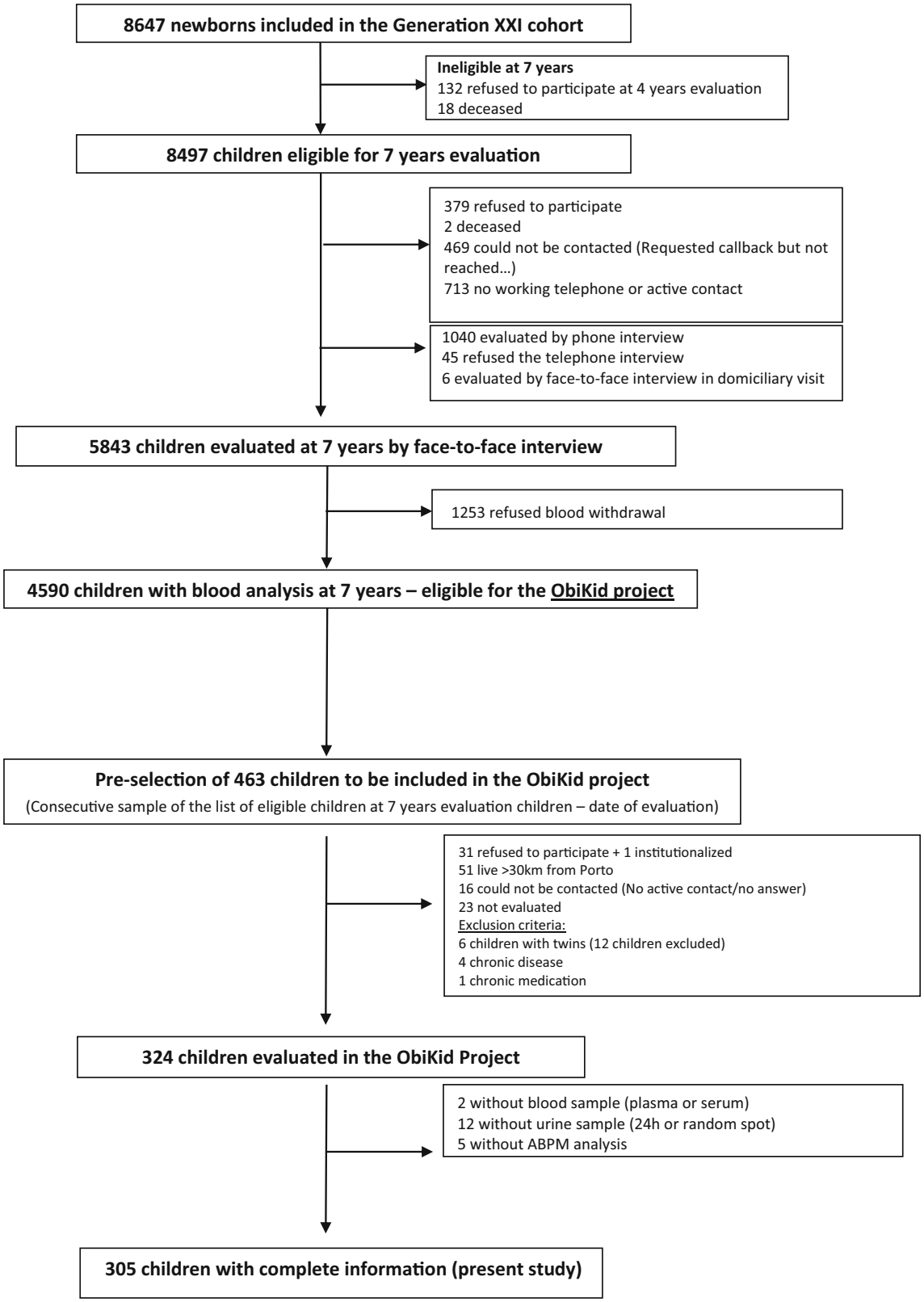

Fig. 1 Patient inclusion

85% with a significance level of 0.05 to detect a difference in estimated glomerular filtration rate (eGFR) of at least 8 ml/min/1.73 m^2 between normal weight and overweight/obese children, assuming a standard deviation (SD) of 24 and

22 ml/min/1.73 m^2 in each group, respectively [29]. We assumed that a minimum of 35% of children would be excluded due to refusal to participate, exclusion criteria, or incomplete information. We needed to consecutively screen and attempt to contact 463 children from the 4590 eligible ones to obtain the required sample; 16 could not be contacted, 32 refused to participate, 23 were unable to schedule study visits during the recruitment period, and 68 met exclusion criteria [four chronic diseases (genetic, renal, or metabolic), one chronic usage of medication (affecting BP, glucose, or lipid metabolism), 51 living far from the study site, and 12 twins]. Participants with acute illness, namely febrile conditions, were asked to postpone the scheduled study visit for a period of at least 15 days after total recovery. We finally enrolled 324 participants, but for our analysis 19 were excluded due to absence of adequate blood or urine samples or missing values in variables of interest. Our study therefore assessed 305 children (Fig. 1). No significant differences were found between included and eligible children with respect to sex, weight, height, systolic/diastolic BP, and parental education level at the follow-up visit at 7 years of age.

Data collection and variable definition

The study visits took place at the Department of Clinical Epidemiology, Predictive Medicine and Public Health, Faculty of Medicine, University of Porto. Data on birth and neonatal characteristics were abstracted from clinical records. Gestational age was considered as determined by ultrasonography. Classes of the sex-specific adequate birth weight for gestational age were defined according to the population-based Canadian reference curves (<10th percentile = small for gestational age; ≥10th percentile and <90th percentile = adequate for gestational age; ≥90th percentile = large for gestational age) [30]. Anthropometric and general physical examinations assessed weight, height, waist circumference and body composition by bioelectrical impedance analysis (percent body fat mass). Data collection was performed according to standard procedures and as previously reported [31]. Waist circumference was indexed to height [waist-to-height ratio (WHtR) in cm/m] for statistical analysis. BMI-for-age values were classified according to the World Health Organization (WHO) growth reference data for BMI z-score into the following categories: normal weight (−2 SD to +1 SD); overweight (> +1 SD), and obesity (> + 2 SD) [32]. To evaluate the effect of duration of obesity on variables studied, we gathered the most recent anthropometric data available from the previous evaluations of the cohort; accordingly, data at 4 years of age was gathered and analyzed. At that age, BMI-for-age values were classified according to the WHO growth reference data for BMI z-score for children <5 years [33]. Among overweight/obese children at the age of 8–9 with anthropometric evaluation at the age of 4 ($n = 132$), two groups were considered: those known to be overweight or obese since the age of 4 ($n = 88$; 39 girls, 49 boys) and those classified as normal weight at the age of 4 ($n = 44$; 18 girls, 26 boys).

Ambulatory BP monitoring (ABPM) was performed for 24 h with a portable, noninvasive, oscillometric BP recorder (Spacelabs Healthcare®, model 90207). The nondominant arm was used to allow free movement during everyday activity and reduce measurement errors due to movement artifacts. Cuff size was chosen according to patient's arm circumference. BP measurements were taken automatically at 20-min intervals during the day and at 30-min intervals during the night. A minimum monitoring duration of 24 h with gaps <2 h was required for acceptance; five of the ABPM analysis examinations were excluded due to insufficient readings. Readings were used to calculate mean 24 h, daytime and nighttime mean arterial (MAP), systolic (SBP) and diastolic (DBP) pressures, using SpaceLabs® software. SD scores for BP values were calculated (least mean square method) according to the published reference values of the German Working Group on Pediatric Hypertension [34]. To characterize circadian BP rhythmicity, we calculated the percentage of nocturnal fall in MAP using the following formula: [mean daytime MAP – mean nighttime MAP]/[mean daytime MAP] × 100. The nondipping pattern was considered when a drop in nighttime MAP <10% of the corresponding daytime BP was observed.

Laboratory procedures

A venous blood sample was collected from all participants after an overnight fast of at least 8 h and analyzed for cystatin C, creatinine, uric acid, aldosterone, and P-AGT. All participants collected a spot urine sample, which was analyzed for albumin, creatinine, and U-AGT, and a 24-h urine sample, which was analyzed for H_2O_2. All standard laboratory measurements were performed in the Clinical Pathology Department of Centro Hospitalar São João, Porto, Portugal. Serum cystatin C was assayed using a particle-enhanced immunonephelometric assay (N latex Cystatin C, Siemens®). Serum creatinine assay was based on the Jaffé compensated traceable to an isotope dilution mass spectrometry method (Olympus AU 5400 automated analyzer, Beckman-Coulter®, USA). Urinary creatinine and albumin were determined with the same clinical immunochemistry analyzer. GFR was estimated by the Zappitelli combined formula (in ml/min/1.73 m^2) [35]. Aldosterone concentration was measured using Liaison Aldosterone Kit® (DiaSorin Inc., USA) in serum samples. Urinary and plasma concentrations of AGT and urinary excretion of H_2O_2 were assessed in the Department of Pharmacology and Therapeutics of the Faculty of Medicine at the University of Porto. AGT was quantified by an immunoenzymatic method, using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Human Total

Angiotensinogen Assay Kit, Immuno-Biological Laboratories Co., Gunma, Japan) and H_2O_2 evaluated using a microplate fluorometric assay (Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Molecular Probes, Alfagene, Carcavelos, Portugal), according to protocols provided by the manufacturers. Data concerning AGT and H_2O_2 have been recently used by our group as part of studies on the same cohort to evaluate the association with urinary fibrogenic cytokines [36] and oxidative stress [37], respectively.

Statistical analysis

Data are presented as mean and SD or, if skewed, as median and 25th–75th percentiles (P25–P75). U-AGT, P-AGT, and U-H_2O_2 had an asymmetric distribution and were logarithmized (base 10) before linear regression analyzes, allowing a normal distribution to be obtained. The tertiles of WHtR, percent of body fat mass, and U-AGT were defined based on all enrolled participants. Linear trend was tested using linear regression models with BMI z-score classes and tertiles of WHtR and body fat mass included as independent continuous variables and U-AGT (logarithm base 10) as the dependent variable. Tertiles of P-AGT were used as independent variables and H_2O_2 (logarithm base 10) as the dependent variable. Models were additionally adjusted for sex and age (in months).

Results

A total of 305 children (54% male) with a mean (SD) age of 8.8 (0.2) years were assessed. General characteristics and levels of biochemical parameters are shown in Tables 1 and 2, respectively, by BMI classes (160 normal weight, 86 overweight, and 59 obese children). Overweight and obese children had higher birth weight but no differences in distribution in classes of adequacy of birth weight for gestational age. Overweight and obese children presented significantly higher values of 24-h and nighttime MAP values (Table 1). Regarding analytical parameters, overweight and obese children presented higher uric acid, creatinine, and cystatin C levels and lower eGFR values. No differences were found in median values of aldosterone, U-albumin, or U-H_2O_2 across groups (Table 2).

The levels of U–AGT were not significantly different across BMI classes (normal weight 5.6 (3.7–7.6); overweight 5.3 (3.4–8.3); obese 4.8 (2.8–7.8) μg/g of creatinine, p = 0.338) and neither were P–AGT levels (normal weight 36.6 (32.4–47.3); overweight 37.3 (32.6–44.8); obese 38.8 (34.1–44.3) μg/ml, p = 0.780) (Table 2). The distribution of U-AGT and P-AGT levels by classes of BMI and tertiles of WHtR and body fat mass is shown in Fig. 2. Regardless of the obesity index considered, no significant differences were found in the linear trend analysis of U-AGT or P-AGT levels (Fig. 2).

No differences were found between sexes in median P-AGT [38.5 (33.6–46.2) vs. 36.3 (31.7–43.4) μg/ml, p = 0.069, in girls and boys, respectively] or U-AGT [5.1 (2.8–8.5) vs. 5.5 (3.7–7.3) μg/g of creatinine, p = 0.370, in girls and boys, respectively] levels. However, in the overweight/obese group, those known to be overweight or obese since the age of 4 were more likely to excrete less U-AGT compared with those classified as normal weight at the age of 4, with this difference being significant only in boys (Table 3). This is indicated by the fact that in boys (but not in girls) who were overweight/obese at the age of 8–9 years only, a markedly higher percent of cases was found in the 2nd and 3rd tertiles of U-AGT, while in boys overweight/obese since the age of 4, the distribution of cases is relatively homogeneous across the three U-AGT tertiles (Table 3).

Linear trend analysis showed that the levels of U-H_2O_2 decreased across P-AGT tertiles in the overweight/obese group [1st tertile: 1577.0 (959.1–2008.9), 2nd tertile: 1328.9 (701.7–2380.1), 3rd tertile: 846.5 (466.0–1609.4) nmol/day, p for trend = 0.039] but not in the normal-weight group [1st tertile: 1007.5 (666.9–2030.7), 2nd tertile: 1323.4 (861.8–2133.4), 3rd tertile: 1522.2 (704.2–2055.3) nmol/day, p for trend = 0.548)] Moreover, among overweight/obese children, the effect of U-H_2O_2 on P-AGT levels was significant in those classified as overweight/obese since the age of 4, but not in those who were not (Fig. 3). The levels of U-H_2O_2 were similar across U-AGT tertiles in children who were overweight/obese at the age of 8–9; in those who were overweight/obese since the age of 4, there was a marginally significant (p = 0.053; Fig. 3) decrease of U-H_2O_2 values across U-AGT tertiles.

Discussion

Previous studies have shown an increase in U-AGT in children with renal disease of different etiologies [16, 38, 39], namely in adolescents with type 1 diabetes [15] or with primary hypertension [14], and in children with very low birth weight [40]. Based on these published data and on the negative impact that obesity has on the kidney [2], we would expect that overweight/obese children had higher U-AGT than age-matched controls of normal weight. However, as recently reported by our group [36], we observed no significant differences in U-AGT between normal and overweight/obese children. In this study, we further extended this result and clarified that it is observed regardless of the obesity index considered: classes of BMI or tertiles of WHtR or percent body fat mass. Although contrary to our initial hypothesis this result in obese but otherwise healthy children is in accordance with that of the only two studies concerning obesity and intrarenal RAAS,

Table 1 General characteristics of study participants by BMI z-score classes

	BMI z-score classification			*P* value
	Normal weight *n* = 160	Overweight *n* = 86	Obese *n* = 59	
General characteristics and anthropometry				
Age (months)	105.1 ± 3.0	105.2 ± 2.8	105.5 ± 2.8	0.663
Male sex	83 (52%)	43 (50%)	40 (68%)	0.069
Gestational age (weeks)	38.8 ± 1.9	38.9 ± 1.3	38.9 ± 1.2	0.891
Birth weight (g)	3185 ± 483	3266 ± 383	3354 ± 442	0.040
Birth weight for gestational age[a]				0.923
Small (<10th percentile)	22 (13.8%)	11 (12.8%)	6 (10.2%)	
Adequate (10th–90th percentile)	133 (83.1%)	72 (83.7%)	50 (84.7%)	
Large (≥90th percentile)	5 (3.1%)	3 (3.5%)	3 (5.1%)	
BMI (kg/m^2)	16.0 ± 1.2	19.5 ± 0.9	23.3 ± 2.4	<0.001
Z-score	−0.04 ± 0.74	1.56 ± 0.30	2.66 ± 0.49	<0.001
WHtR (cm/m)	44.7 ± 2.6	50.2 ± 3.2	56.6 ± 4.4	<0.001
Percent body fat mass	10.6 ± 7.1	20.0 ± 8.0	27.7 ± 9.4	<0.001
24-h ambulatory BP				
24-h MAP (mmHg)	81.1 ± 4.4	82.5 ± 5.2	82.9 ± 6.3	0.030
Z-score	0.35 ± 0.90	0.69 ± 1.07	0.51 ± 0.92	0.032
Daytime MAP (mmHg)	84.8 ± 4.6	85.8 ± 5.8	86.2 ± 6.5	0.157
Z-score	0.06 ± 0.86	0.28 ± 0.96	0.15 ± 0.96	0.219
Nighttime MAP (mmHg)	73.4 ± 4.9	74.8 ± 5.2	75.6 ± 6.3	0.011
Z-score	0.52 ± 0.91	0.85 ± 0.94	0.72 ± 0.79	0.022
MAP dipping (%)	13.4 ± 4.7	12.6 ± 5.2	12.3 ± 4.5	0.212
Nondipping pattern	34 (22%)	30 (35%)	17 (29%)	0.082

Mean ± standard deviation or *n* (%). BMI z-score classification is according to WHO criteria (normal weight, overweight, and obesity) [32]

BMI body mass index, *WHtR* waist-to-height ratio, *MAP* mean arterial pressure

[a] According to the population-based Canadian reference curves [30]

which reported similar U-AGT between obese and nonobese adults [7, 18]. In contrast, animal models of obesity show an imbalance of renal RAAS components. Obese animals have increased expression of angiotensin-converting enzyme (ACE) and of AT_1 and AT_2 receptors, but decreased expression of ACE2 and Mas receptor [41]. Adipose-derived AGT determines P-AGT concentration that, by controlling circulating AngII levels, stimulates proximal tubule production of AGT [11], thus activating intrarenal RAAS activity. Although plasma levels of AGT are increased in obese hypertensive compared with nonobese hypertensive patients [42], in our population, P-AGT was similar between normal and overweight/obese children, whatever obesity index was considered. The fact that we found similar levels of U-AGT and P-AGT might be associated with the duration of obesity (i.e., that might be not enough to have a negative impact on kidney function), activation of slow operating compensatory mechanisms, or both. Interestingly, it has been reported that obesity severity, duration, and age at onset might be crucial for the development and progression of obesity-related organ damage. Using a contemporary population from the Framingham study, Abdullah et al. suggested that a construct of obese-years should be preferable to BMI or duration of obesity alone to estimate the obesity-associated risk of type 2 diabetes [20]. Also, an adolescent onset of obesity was found to have a stronger impact on diabetes risk than adult-onset obesity [19]. When we analyzed our group of overweight/obese children according to the duration of overweight/obesity, we observed those with a longer duration presented lower levels of U-AGT than those with recent offset of overweight/obesity. This was quite unexpected given that if renal RAAS progressively contributes to renal damage, along with the duration of excessive weight, then children already overweight/obese by the age of 4 would show higher U-AGT. Some children in our population were identified as being obese at the age of 4 but as being of normal weight at the age of 8–9; however, this group was too small to be analyzed.

Interestingly, we previously reported [28] an already lower eGFR in these overweight/obese children, although renal function was in the normal range for all children. Also, we

Table 2 Laboratory data by BMI z-score classes

	BMI z-score classification			*P* values
	Normal weight *n* = 160	Overweight *n* = 86	Obese *n* = 59	
Blood analytical parameters				
Uric acid (mg/dl)	3.5 ± 0.7	3.8 ± 0.7	4.0 ± 0.8	<0.001
Creatinine (mg/dl)	0.43 ± 0.06	0.45 ± 0.06	0.45 ± 0.06	0.003
Cystatin-C (mg/L)	0.64 ± 0.07	0.67 ± 0.08	0.68 ± 0.06	<0.001
eGFR (ml/min/1.73 m^2)	138.4 ± 15.8	132.2 ± 16.4	132.1 ± 13.2	0.003
Aldosterone (ng/dl)	9.4 (7.1–13.2)	12.1 (7.5–16.2)	9.6 (8.4–13.8)	0.216
P-AGT (μg/ml)[a]	36.6 (32.4–47.3)	37.3 (32.6–44.8)	38.8 (34.1–44.3)	0.780
Urinary analytical parameters				
U-albumin (mg/g creatinine)	4.1 (2.6–7.6)	3.6 (2.1–7.8)	3.7 (2.3–5.6)	0.560
U-AGT (μg/g creatinine)[a]	5.6 (3.7–7.6)	5.3 (3.4–8.3)	4.8 (2.8–7.8)	0.338
U-H_2O_2 (nmol/day)[b]	1282.0 (711.1–2058.7)	1328.9 (680.5–1988.5)	1276.2 (701.7–2187.2)	0.855

Mean ± standard deviation or median (P25–P75). BMI z-score classification is according to WHO criteria (normal weight, overweight, and obesity) [32]

BMI body mass index, *eGFR* estimated glomerular filtration rate by Zappitelli combined formula, *P-AGT* plasma angiotensinogen, *U-albumin* urinary albumin-to-creatinine ratio, *U-AGT* urinary angiotensinogen, *U-H_2O_2* urinary hydrogen peroxide

Data concerning AGT ([a]) and H_2O_2 ([b]) have been recently used by our group as part of studies on the same cohort intended to evaluate the association with urinary fibrogenic cytokines [36] and oxidative stress [37], respectively

recently reported [36] that urinary excretion of the fibrogenic cytokines endothelin-1 (ET-1) and transforming growth factor-β1 (TGF-β1) was not increased in obese children in our cohort; indeed, they showed lower levels of urinary ET-1 and TGF-β1 than those of normal weight. Furthermore, U-AGT was associated with urinary excretion of ET-1 and TGF-β1 [23]. Of note, although there are reports of human studies showing increased expression of AGT messenger RNA (mRNA) in subcutaneous adipose tissue of obese individuals [43], most human and animal studies report a decrease [9, 12, 44]. Moreover, expression and secretion of AGT decreases as adipocytes become hypertrophied. This effect seems to be, at least in part, mediated by reactive oxygen species, namely, H_2O_2 [12]. Consistent with this, we observed an inverse association between P-AGT and U-H_2O_2 in overweight/obese children but not in normal-weight controls. Moreover, that association was seen only in children overweight/obese by the age of 4. So, although further studies are needed to fully evaluate and characterize this point, our results suggest that childhood obesity is associated with time-dependent alterations in intrarenal RAAS activity (even when established markers of renal function are in the normal range) and with an association between P-AGT and U-H_2O_2.

It is noteworthy that the difference observed in U-AGT according to duration of obesity was only significant in boys. In girls, the levels of U-AGT were not associated with the duration of overweight/obesity . Studies in animal models suggest that the kidney has estrogen receptors that can mediate protective effects against renal injury by attenuating glomerulosclerosis and tubulointerstitial fibrosis [45, 46]. Interestingly, this protection has been suggested to involve both the RAAS and the endothelin system [45]. The children in our study are at a prepubertal age and we were not able to quantify neither Tanner stages nor sex hormones. As such, although we recruited a homogeneous population, we cannot conclude whether prepubertal girls already have higher levels of estrogens and androgen metabolites than prepubertal boys, as reported elsewhere [47, 48]. Moreover, the fact that obesity might anticipate puberty in girls and delay it in boys [49] could somehow blunt the impact of obesity on U-AGT levels. In other words, the absence of difference in U-AGT according to duration of obesity in girls could also be explained by the fact that girls in the obese group were at a more advanced phase of pubertal development. In line with this, we also observed that boys excreted significantly more H_2O_2 than girls (unpublished observations) and that in boys, higher salt consumption was associated with higher systolic BP, specifically in those overweight/obese [50].

Major strengths of our study are inclusion of a large and homogeneous sample of healthy prepubertal children and the gathering of data on 24-h ABPM and renal function from all of them. The cross-sectional design of our study might be seen as a limitation. However, as our cohort will be followed until adulthood, we expect to re-evaluate these children in the next evaluation period, when they will be adolescents, and it will

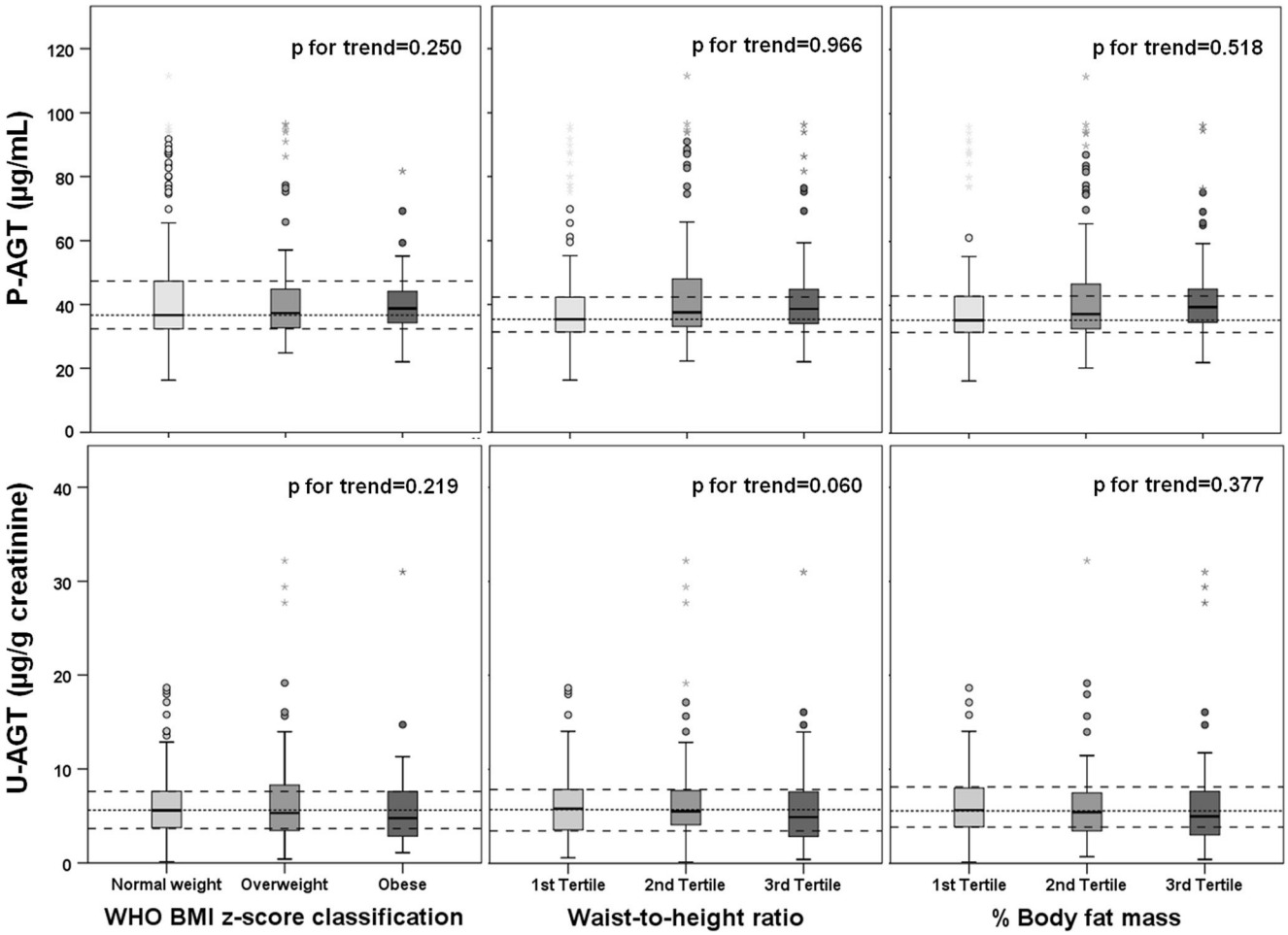

Fig. 2 Distribution of P-AGT and U-AGT by classes of BMI and tertiles of waist-to-height ratio and percent body fat mass. Normal weight, overweight, and obesity group classification is according to the WHO classification for BMI z-score [32]. Tertiles of waist-to-height ratio (≤45.65; 45.66–50.00; >50.00) and percent of body fat mass (≤11.10; 11.11–20.70; >20.70) were divided based on all enrolled participants. P-AGT and U-AGT data is expressed as medians and percentiles 25 and 75. *P* values for linear trend across groups were calculated by linear regression using U-AGT as dependent variable (logarithm base 10) and adjusting for sex and age in months. *Horizontal dashed lines* represent the 25th, 50th, and 75th percentiles in the normal-weight group for each parameter. *WHO* World Health Organization, *BMI* body mass index, *P-AGT* plasma angiotensinogen, *U-AGT* urinary angiotensinogen

be possible to perform a longitudinal analysis to complement and expand the hypothesis raised in this study. In addition, longitudinal studies should include a group of children that has experienced weight loss to test whether that would protect them from metabolic changes later in life.

Our results reinforce the importance of encouraging preventive measures targeting obesity even in prepubertal children, since obesity seems to have an impact on kidney function even at this young age. Moreover, data on sex hormones and Tanner stages would have enriched our analysis significantly.

Table 3 Distribution of cases (%) across urinary excretion of angiotensinogen (U-AGT) tertiles

	U-AGT (µg/g creatinine)		
	1st tertile	2nd tertile	3rd tertile
Boys ($p = 0.036$)			
Overweight/obese since the age of 4	36.7%	34.7%	28.6%
Overweight/obese only at the age of 8–9	11.5%	61.5%	26.9%
Girls ($p = 0.383$)			
Overweight/obese since the age of 4	48.7%	12.8%	38.5%
Overweight/obese only at the age of 8–9	38.9%	27.8%	33.3%

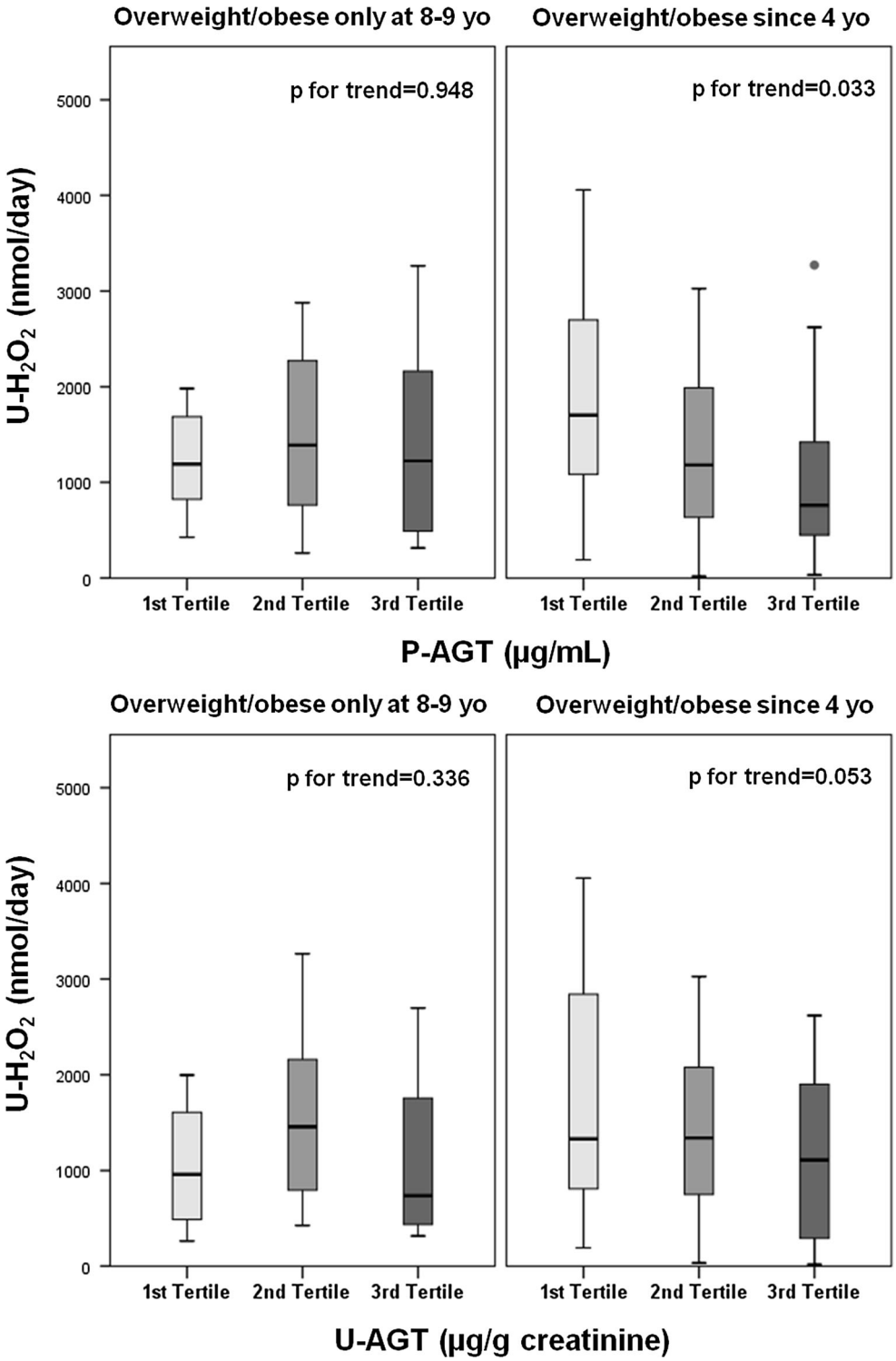

Fig. 3 Distribution of U-H_2O_2 by tertiles of P-AGT and U-AGT, according to duration of overweight/obesity. Tertiles of P-AGT (≤35.0; 35.0–41.5; >41.5) and U-AGT (≤4.1; 4.1–6.7; >6.7) were divided based on all enrolled participants. U-H_2O_2 data is expressed as medians and percentiles 25 and 75. *P* values for linear trend across groups were calculated by linear regression using U-H_2O_2 as dependent variable (logarithm base 10) and adjusting for sex and age in months. *WHO* World Health Organization, *BMI* body mass index, *U-H_2O_2* urinary hydrogen peroxide, *P-AGT* plasma angiotensinogen, *U-AGT* urinary angiotensinogen

Alternatively, studies in prepubertal children should be conducted at even youger ages so that the differences in hormonal levels would not bias the interpretation of the associated results. Certainly, future analysis by our group will pursue this issue.

In conclusion, although intrarenal RAAS activity in overweight and obese children was similar to that of a normal-weight control group, overweight/obese boys —but not girls— with a longer duration of obesity presented lower levels of U-AGT and an inverse association between U-H_2O_2 and P-AGT. This might tempt one to think that ACE inhibitors or angiotensin receptor bolckers (ARBs) could be less effective in protecting the kidney of obese patients. However, this study is

the first to address the role of the RAAS in the context of obesity-induced renal damage. Also, it focused on short-lasting, early childhood obesity, which might not have the same consequences as chronic obesity. Therefore, further studies are essential (including longitudinal studies) to support evidenced-based guidelines for obese patients, since this population has only been addressed in expert opinion and not in actual guidelines.

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Compliance with ethical standards The Generation XXI study was approved by the Ethics Committee of Centro Hospitalar São João, E.P.E. and Faculty of Medicine of the University of Porto, Portugal, and by the National Data Protection Commission. It complies with the Helsinki Declaration and the current national legislation. Written informed consent from parents (or their legal substitute) and verbal assent from children was obtained, concerning information and biological samples gathering.

Conflicts of interest None of the authors have any financial or nonfinancial competing interests concerning the present study.

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